Clinical trial for accelerating the fecal excretion of dioxins

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Introduction

We have previously reported that the level of PCDF in the subcutaneous adipose tissue of Yusho patients is 100 times higher than that in normal subjects, and that the fecal PCDF excretion by these patients corresponds to its concentration in subcutaneous adipose tissue as well as in blood (1). Removal of the toxic chemicals that still remain in the body is thought to be the most effective therapy for the disease. Takenaka *et al*, first observed that in a group of rats fed a diet containing 10% rice bran fiber (RBF) and cholestyramine (CHO), the fecal excretion of PCB increased 5.4 times that seen in the control group (2). In view of the findings, a clinical trial of the combination of RBF and CHO was carried out to promote the excretion of PCDF retained in Yusho patients. Upon administration, a tendency of excess fecal excretion was observed, but the effect of the therapy could not be confirmed (3). In the present study, a similar trial was carried out on Yu-Cheng patients in Taiwan to examine the degree of enhancement of excretion of residual dioxins and PCB.

Materials and Methods

Chemicals

Cholestyramine obtained form Bristol Myers Co. Ltd. and RBF refined by the Prosky method (4) containing 85% dietary fiber (23.5% cellulose, 43.2% hemicellulose and 18.4% lignin) were used. All other chemicals used were commercially available.

Administration of drugs and collection of stool samples

Six Yu-Cheng patients were orally administered 6 g of RBF and 4 g of cholestyramine suspended in a cup of water three times a day after meals for 2 weeks. For the experiment, all stool samples excreted were collected weekly. Twenty milliliters of blood was obtained from each patient before and after the experiment.

Determination of dioxins and PCB

Blood sample

For the determination of PCB in the blood, 2 ml of 1 N KOH-ethanol was added to a portion of sample (2 g). The mixture was refluxed at 80 °C for 1 hour, and then extracted 3 times with hexane. The extract was cleaned up by a silica gel column, and analyzed with ECD/GC. For dioxin determination in the blood, a portion of the sample (10 g) was extracted 3 times with acetone/hexane (2:1, v/v), and after the addition of distilled water, the hexane layer was collected. Then, the extract was concentrated to dryness and weighed. The extract was dissolved with hexane and was cleaned up with a silver nitrate-silica gel column, charcoal column, Florisil column and concentrated sulfuric acid, and analyzed with HRGC/HRMS.

Stool sample

Stool samples of each patient were pooled together for each period separately (7-days before the administration, 6-days from the beginning of the administration, and the following 7-days). Samples for each period were homogenized. For the determination of dioxins and PCB, 100g of each sample was extracted 3 times with chloroform/methanol (1:1, v/v). The extracts were filtered through glass filter paper, and after dilution with distilled water, the chloroform layer was collected. The chloroform layer was concentrated to dryness and weighed as lipid. A portion (20 ml) of 1N KOH-ethanol was added to the extracts, it was mixed and left at room temperature over night, and extracted with hexane. The hexane extracts were concentrated and adjusted to 10 ml.

A portion (2 ml) of hexane solution was cleaned up with silica gel column for the determination of PCB with ECD-GC, and another portion of the solution (8 ml) was cleaned up with concentrated sulfuric acid, a silver nitrate-silica gel column, charcoal column and Florisil column for the determination of dioxins with HRGC/HRMS.

Determination of cholestyramine excreted into stools

One to four grams of stool were weighed into 50 ml centrifuge tube, 2N KOH-ethanol was added and then refluxed at 80 ºC for 1 hour. After the mixing with distilled water, they were centrifuged at 2000 rpm. The water-ethanol supernatant was removed and the pellet was washed with acetone. Subsequently, 2.5% of NaOCl was added to the residue and was left at room temperature overnight. Distilled water was added and the mixture was centrifuged at 2000 rpm.

The water supernatant was removed and the pellet was washed with acetone. Subsequently, cold 72% of sulfuric acid was added to the pellet and mixed, and then it was left at 4 ºC overnight. Finally, the mixture was filtered with a G-4 glass filter and the trapped material was washed with distilled water, hot water, acetone, and hexane respectively. G-4 glass filter and the trapped material were let to dryness at 105 ºC for 4 hours and after cooled they were weighed (A). Then, they were heated at 525 °C for 4 hours, and weighed after cooled (B). The difference in weight between A and B was calculated as the weight of cholestyramine.

Results and Discussion

Dioxins and PCB in the blood

Table 1 shows the concentrations of PCDD, PCDF, coplanar PCB (CoPCB) and PCB in the blood obtained from each patient before and after the clinical trial on a whole base. After the clinical trial, each average concentrations were found to decrease for most of the determined congeners as compared with those before the trial. The decrease seen in the concentration of 1,2,3,6,7,8-HxCDD, PeCDF or total TEQ was each stastically significant ($p<0.01$) and the decrease in fat concentration seen was also significant ($p<0.05$).

Dioxins and PCB excretion into the stool before and after the administration of RBF and CHO

Table 2 shows the fecal excretion of PeCDF (2,3,4,7,8-PeCDF), HxCDF (1,2,3,4,7,8- and 1,2,3,4,7,8-HxCDF) and PCB into the stools during the experiment. Stool weight was

Table 1 Concentrations of PCDD, PCDF, CoPCB and PCB in blood of Yu-Cheng patients (ppt, whole base)

	Patients								
Congener	A01	A02	A03	D04	D05	D12	D14	D15	Mean
2,3,7,8-TCDD	ND	0.02	0.03	ND	ND	0.01	0.03	ND	0.02
	0.02	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
1,2,3,7,8-PeCDD	0.05	0.07	0.13	0.02	0.04	0.04	0.04	0.03	0.05
	0.04	0.03	0.08	0.03	0.03	0.03	0.03	0.04	0.04
1,2,3,6,7,8-HxCDD	0.31	0.42	0.92	0.18	0.33	0.36	0.36	0.44	0.41
	0.28	0.33	0.72	0.17	0.22	0.18	0.26	0.39	0.32
1,2,3,4,6,7,8-HpCDD	0.21	0.13	0.29	0.23	0.21	0.25	0.15	0.17	0.21
	0.24	0.14	0.24	0.14	0.13	0.15	0.14	0.19	0.17
OCDD	1.7	2.9	4.6	1.3	1.1	2	1.8	1.6	2.1
	1.8	2.4	4	1	1.7	1.4	1.4	1.5	1.9
2,3,7,8-TCDF	0.05	0.07	0.06	0.02	0.04	0.03	0.03	0.03	0.04
	0.03	0.03	0.03	0.02	0.03	0.06	0.04	0.03	0.03
1,2,3,7,8-PeCDF	0.05	0.06	0.08	0.05	0.03	0.04	0.02	0.03	0.04
	0.04	0.02	0.04	0.02	0.02	0.06	0.03	0.04	0.03
2,3,4,7,8-PeCDF	2.6	3.5	3.8	1.3	1.9	1	1.9	2.7	2.3
	2	3	2.8	1	1.3	0.5	1.2	2.3	1.8
1,2,3,4,7,8-HxCDF	5.4	9.7	15.8	3.8	5.2	5.3	4.7	8.7	7.3
	4.9	10.8	13.1	3.1	4	3.3	4.2	10.1	6.7
1,2,3,4,6,7,8-HpCDF	0.19	0.4	0.44	0.16	0.21	0.17	0.13	0.16	0.23
	0.2	0.28	0.32	0.16	0.13	0.2	0.24	0.2	0.22
3,3',4,4'-TCB	0.1	0.16	0.12	0.07	0.06	0.07	0.09	0.05	0.09
	0.17	0.06	0.09	0.06	0.05	0.13	0.1	0.09	0.09
3,3',4,4',5-PeCB	0.21	0.21	0.53	0.21	0.22	0.34	0.34	0.96	0.38
	0.31	0.14	0.5	0.19	0.17	0.29	0.26	0.65	0.31
3,3',4,4',5,5'-HxCB	0.16	0.13	0.41	0.07	0.09	0.18	0.14	0.36	0.19
	0.2	0.11	0.45	0.08	0.11	0.14	0.14	0.31	0.19
Total TEQ [a]	2	2.9	3.8	1.1	1.6	1.2	1.6	2.5	2.1
	1.6	2.7	2.9	0.9	1.2	0.7	1.1	2.3	1.7
PCBs(ng/g)	11	10	45	13	23	18	21	64	26
	11	9	32	12	22	19	13	38	20
Fat(%)	0.46	0.43	0.8	0.34	0.37	0.54	0.44	0.48	0.48
	0.38	0.4	0.56	0.33	0.33	0.41	0.39	0.4	0.4

a: Calculated based on the TCDD equivalent factors as determined by NATO for PCDDs and PCDFs, and WHO for Co-PCBs.

The data on upper line show the concentrations in the blood collected in January 1993 (before the clinical trials) while those on lower line show the concentrations in the blood collected in August 1994 (after the clinical trials) .

212±45 g/day before the administration, 245±50 g/day during the first week of the administration, and 215±61 g/day during the second week. The fecal excretion of PeCDF in each period were 837±449, 919±516 and 945±461pg/day, respectively. The fecal excretion of HxCDF were 2894±1421, 2556±1183 and 2536±1006pg/day, respectively. On the other hand, the fecal excretion of PCB were 565±267, 716±394 and 613±223ng/day, respectively. These findings show that the amount of stools and the fecal PeCDF and PCB excretion were slightly increased with the administration of RBF and CHO. However, the fecal excretion of HxCDF was less than that before treatment.

Table 2 Fecal PCDF and PCB excretion of Yu-Cheng patients

Patient	Administration	Stool (g/day)	PeCDF (pg/day)	HxCDF (pg/day)	PCB (ng/day)
	Before	280	1027	2822	504
A01	First week	304	976	2518	601
	Second week	328	1134	2633	645
	Before	217	1311	3323	415
A02	First week	236	1608	3350	497
	Second week	210	1459	2875	394
	Before	214	902	4103	536
A03	First week	202	840	2897	589
	Second week	230	1203	3463	575
	Before	209	427	1443	384
D04	First week	297	520	1327	466
	Second week	188	465	1303	432
	Before	210	1189	4615	1098
D05	First week	224	1358	4159	1510
	Second week	181	1120	3599	1020
	Before	139	166	1055	453
D12	First week	180	214	1083	634
	Second week	155	286	1341	614

Table 3 Determination of CHO in the stools of Yu-Cheng patients (g/day)

Patient	A01	A02	A03	D04	D05	D12
Before administration	0.01	0.2	0.06	0.3	0.1	0.1
First week	15.4	7.9	12.3	8.7	6.5	9.8
Second week	14.6	9.2	11.7	3.9	3.6	9.3

Table 4 Calibrated data of PCDF and PCB fecal excretion of Yu-Cheng patients by the cholestyramine in the stools

Patient	Administration	Stool (g/day)	PeCDF (pg/day)	HxCDF (pg/day)	PCB (pg/day)
	Before	280	1027	2822	504
A01	First week	304	976	2518	601
	Second week	328	1134	2633	645
	Before	217	1311	3323	415
A02	First week	361	2458	5121	760
	Second week	274	1903	3750	514
	Before	214	902	4103	536
A03	First week	202	840	2897	589
	Second week	230	1203	3463	575
	Before	209	427	1443	384
D04	First week	410	717	1830	643
	Second week	578	1431	4009	1329
	Before	210	1189	4615	1098
D05	First week	413	2507	7678	2788
	Second week	603	3733	11997	3400
	Before	139	166	1055	453
D12	First week	222	263	1333	780
	Second week	201	371	1740	797

CHO excreted into the stools

Table 3 shows the amount of CHO excreted into daily stools of Yu-Cheng patients during the experiment. CHO is an anion exchange resin, and it is not absorbed or metabolized. Furthermore, over 96% of CHO was excreted into feces within 24 hours in rat. As 12 g/day CHO was administered to all the patients, it is obvious that the same amounts should be excreted into the stools. In patient A01, the amount regarded as CHO was 15.35 g/day in the first week, and 14.55 g/day in the second week of the administration. At present, we have no explanation

regarding the large amount of CHO. In patient A03, the amount regarded as CHO coincided with 12 g. In patients A02, D04, D05 and D12, the amount ranged from 3.6 to 9.8 g/day. It is suggested that the patients had failed to collect all the stools they excreted. The data in Table 2 was recalibrated according to the data shown in Table 3, and show in Table 4.

Figure 1 shows the increase in the excretion of PeCDF, HxCDF and PCB into the stool compared with that before the treatment. In patients A02, D04, D05 and D12, the increase were from 60 to 160% for PeCDF, 30 to 110% for HxCDF, and 50 to 190% for PCB. However, these excretions were not increased in patients A01 and A03 regarding PeCDF, HxCDF and PCB. Administration of 18 g/day of RBF, and 12 g/day of CHO for two weeks caused an increase in excretion of PeCDF, HxCDF and PCB from 30 to 190% in four out of six Yu-Cheng patients.

These findings had been reported in 14th International Symposium on Chlorinated Dioxins, PCB and Related Compounds, Nov., 1994 in Kyoto, Japan. Most time have passed from the presentation. Recently, Morita et al. (5, 6) and Aozasa et al. (7) reported various dietary fibers, chlorella and chitin increased the fecal excretion of dioxins in animal experiments. Nagayama *et al.* (8) reported the decrease in PCDF level of human blood after the one year intake of FBRA (brown rice e fermented with *Aspergillus oryze*). We preliminarily obtained an information that the volunteer's blood levels of dioxins were not varied when boiled spinach (150 g/day) was continuously eaten for 6 months. As the mentioned above, the acceleration of dioxins elimination is remarkably effective in these animal experiments. The investigation of the human intervention does not identify the acceleration of the dioxins elimination from human body burden. On the other hand, Geusau *et al.* (9) reported that the fecal elimination of TCDD was elevated from 8 to 10 times at the high Olestra dose (10g). This finding indicate that the trial was effective at the acute stage of toxicants poisoning, such as dioxins. However, Yusho and Yu-Cheng patients are chronic stage, and these trials might have no effect, except for the typical patients. Since the occurrence of Yusho, over 30 years have passed. We are performing the follow-up research of dioxins in about 83 patients blood from 1995. Ministry of Health, Labour and Welfare, Japan announced the follow-up research of dioxins in all Yusho patients to request the dioxins analysis in their blood. We improved the dioxins analytical protocol in human blood, which is ten times more sensitive than conventional methods. This method consists with an extraction of lipids from blood by accelerate solvent extraction system, a reduction of agent blank by downsizing of column cleanup and a large volume injection of sample to HRGC/HRMS. By this method, we

could determine the dioxins from only 5ml of blood, and we could easily make the sample preparation from blood in dioxins analysis. Therefore, with this method it will be possible to analyze dioxins from many samples extracted from blood in a short time. Follow-up research of all Yusho patients is due to be conducted by request of the Ministry of Health, Labour and Welfare, Japan in the near future. When the results of this research are analyzed, the correlation between the dioxin concentration, which remains in the human body and various kinds of symptoms would become clear, and could be, performed suitable risk management of dioxins damage. We hope that better therapies of dioxins damage are developed in the future.

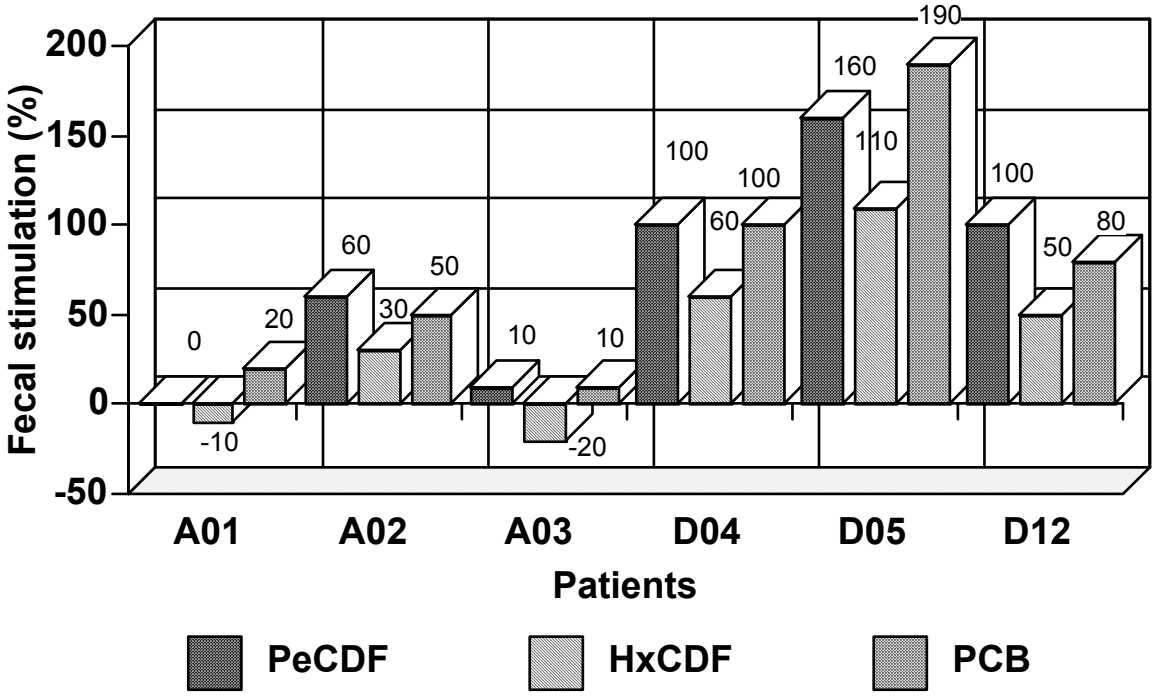

Figure 1 Stimulation of PeCDF, HxCDF and PCB in the stools by the administration of RBF and CHO

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